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Pollutant particles produce vasoconstriction and enhance MAPK signaling via angiotensin type I receptor

Zhuowei Li[1], Jacqueline D. Carter[2], Lisa A. Dailey[2], Yuh-Chin T. Huang[2]

[1]Center for Environmental Medicine, Asthma and Lung Biology

University of North Carolina, Chapel Hill, NC 27599

and

[2]National Health and Environmental Effects Research Laboratory

Office of Research and Development

Environmental Protection Agency, Research Triangle Park, NC 27711

Correspondence: Yuh-Chin T. Huang, M.D., M.H.S.

CB 7315, 104 Mason Farm Road

Chapel Hill, NC 27599

919-843-9504

FAX: 919-966-6271

E-mail: huang.tony@epa.gov

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Abbreviations: angiotensin type 1 receptor (AT_1R), particulate matter (PM), human pulmonary artery endothelial cells (HPAECs), nitric oxide (NO), epidermal growth factor receptor (EGFR), angiotensin converting enzyme (ACE), mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinases ½ (ERK1/2).

ABSTRACT

Exposure to particulate matter (PM) is associated with acute cardiovascular mortality and mobidity, but the mechanisms are not entirely clear. In this study, we hypothesized that PM may activate the angiotensin type 1 receptor (AT_1R), a G protein-coupled receptor that regulates inflammation and vascular function. We investigated the acute effects of St. Louis urban particle (UP, SRM1648) on the constriction of isolated rat pulmonary artery rings and the activation of extracellular signal-regulated kinases1/2 (ERK1/2) and p38 mitogen-activated protein kinases (MAPKs) in human pulmonary artery endothelial cells (HPAECs) with or without losartan, an antagonist of AT_1R. UP at 1-100 μg/ml induced acute vasoconstriction in pulmonary artery. UP also produced a time- and dose-dependent increase in phosphorylation of ERK1/2 and p38 MAPK. Losartan pretreatment inhibited both the vasoconstriction and the activation of ERK1/2 and p38. The water-soluble fraction of UP was sufficient for inducing ERK1/2 and p38 phosphorylation, which was also losartan-inhibitable. Copper and vanadium, two soluble transition metals contained in UP, induced pulmonary vasoconstriction and phosphorylation of ERK1/2 and p38, but only the phosphorylation of p38 was inhibited by losartan. The UP-induced activation of ERK1/2 and p38 was attenuated by captopril, an angiotensin-converting enzyme inhibitor. These results indicate that activation of the local renin-angiotensin system may play an important role in cardiovascular effects induced by PM.

INTRODUCTION

Over the last decade, a growing body of epidemiological and clinical evidence has raised the possibility of the potentially deleterious effects of ambient pollutant particles on cardiovascular health. Exposure to particulate matter (PM) has consistently been associated with increased hospitalization and mortality due to cardiovascular diseases (Pope et al. 2004). It has been estimated that for each 10-$\mu g/m^3$ increase in PM_{10} (particulate matter < 10 μm in aerodynamic diameter), the daily cardiopulmonary mortality increased by 0.3% (Dominici et al. 2003). The risk is especially high in patients with congestive heart failure, frequent arrhythmias, or both (Goldberg et al. 2001; Mann et al. 2002), and the catastrophic cardiac events may occur as early as hours after PM exposure (Peters et al. 2001). The mechanisms for the acute increase in cardiovascular events are not entirely clear. Several hypotheses have been proposed, including imbalance in autonomic systems, increases in pro-coagulant activities and systemic release of inflammatory mediators (Brook et al. 2004; Donaldson et al. 2001).

Recent *in vivo* and *in vitro* evidence also indicate that PM may cause endothelial dysfunction and vasoconstriction. Exposure to concentrated ambient particles (CAPs) (median, 182.75 $\mu g/m^3$) for 5 h/day for 3 days decreased the lumen/wall area ratio of small pulmonary arteries in rats indicating increased pulmonary vascular resistance (Batalha et al. 2002). Motorcycle exhaust particulate enhanced constriction of rat aortic rings induced by phenylephrine (Tzeng et al. 2003). Exposure to PM for 4 weeks increased atherosclerotic plaque formation in rabbits (Suwa et al. 2002). Inhalation of CAPs (~150 $\mu g/m^3$) and ozone (120 ppb) for 2 hours causes acute constriction of brachial

artery in healthy adults (Brook et al. 2002). An air pollution episode in Germany was associated with increases in systemic blood pressure by as much as 8 mmHg (Ibald-Mulli et al. 2001). Various vasoconstrictor mechanisms have been demonstrated, including the release of endothelins (Bouthillier et al. 1998; Thomson et al. 2004), activation of the epithelial growth factor receptor (EGFR) (Huang et al. 2002), and inhibition of nitric oxide (NO) production (Bai et al. 2001; Bouthillier et al. 1998; Huang et al 2002; Ikeda et al. 1995). These mechanisms, however, could not completely explain the epidemiological findings of the acute effects of PM on cardiovascular events, which have a lag time of hours. The endothelins are potent vasoconstrictors, but the increased release occurs 24 hours after PM exposure. Vasoconstriction caused by the activation of EGFR is relatively weak, and the inhibition of NO production results in a loss of vasodilator activity.

The circulating and local renin-angiotensin systems have been known to play a key role in the pathogenesis of cardiovascular diseases (Dzau 1988). The end-product of this pathway, angiotensin II, is one of the most potent vasoconstrictors, and its effects are mediated primarily by the G protein-coupled angiotensin type 1 receptor (AT_1R) (Daugherty and Cassis 2004). Agonist binding of AT_1R activates MAPKs (Touyz and Schiffrin 2000), a common early signaling event induced by PM exposure (Roberts et al. 2003; Silbajoris et al. 2000). In the preliminary experiments, we found that pulmonary vasoconstriction induced by St. Louis urban particle (UP, SRM1648) could be inhibited by losartan, an AT_1R antagonist. In the present study, we characterized the role of AT_1R in UP-induced vasoconstriction and MAPK activation. The study was performed in the

isolated pulmonary artery (PA) ring system and human pulmonary artery endothelial cells (HPAECs).

MATERIALS AND METHODS

Reagents and Chemicals. HPAECs were obtained from Cell Applications, Inc. (San Diego, CA). Endothelial growth medium (EGM-2) and supplements were from Clonetics (Bio Whittaker Inc., Walkersville, MD). Vanadyl sulfate ($VOSO_4$) and copper sulfate ($CuSO_4$) were from Johnson Matthey Co. (Ward Hill, MA). Captopril was from Sigma Chemical Co. (St. Louis, MO). SB203580 and PD98059 were obtained from Calbiochem-Novabiochem Corp. (San Diego, CA). Losartan potassium was from Merck &Co., Inc. (West Point, PA). Monoclonal antibodies against phospho-p38, total p38 phospho-ERK1/2 and total ERK1/2 were purchased from Cell Signaling Technology, Inc. (Beverly, MA). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit and goat anti-mouse IgG were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Molecular mass standards, polyacrylamide and buffers were from Bio-Rad (Richmond, CA). The ECL western blotting detection reagents were purchased from Amersham Biosciences Corp. (Piscataway, NJ).

Urban Particles: St. Louis urban particles (SRM 1648) were purchased from the National Institute of Standards and Technology (Gaithersburg, MD). They were prepared from urban PM collected in the St. Louis, MO area in a baghouse over a period of over 12 months. The material was removed from the filter bags, combined in a single lot, screened through a fine mesh sieve to remove extraneous materials and thoroughly blended in a v-blender. The material was then packaged into sequentially numbered bottles. The major constituent elements are (mass fraction in %): Al 3.4%, Fe 3.9%, K 1.1%, and the minor constituent elements are: Na 0.43%, Pb 0.66%, Zn 0.48%. There are

also trace constituents (ng/mg), including As (115), Cd (75), Cr (403), Cu (609), Mn (786), Ni (82), Se (27), U (5.5) and V (127). UP was suspended in distilled deionized water for the use in experiments.

Preparation of the water soluble and insoluble fraction of UP. The water suspension of UP was centrifuged for 10 min at 14,000 rpm. The supernatant was collected and centrifuged again. This procedure was repeated several times until no sediments could be seen. This final supernatant was designated as the water-soluble fraction of UP. The pellets from the first centrifuge were washed using deionized distilled water for three times and then resuspended in appropriate volume of water. This was designated as the water-insoluble fraction of UP.

Isolated perfused rat pulmonary artery (PA) ring model. Segments of the right and left main pulmonary arteries of Sprague-Dawley rats (250-350 g) measuring approximately 2-3 mm were removed and placed in the Krebs-Henseleit (KH) buffer as described previously (Li et al. 2004). The artery segments were then suspended in the Radnoti 4-unit tissue organ bath system (Glass Technology Inc., Monrovia, CA). The reservoirs held 20 mL of KH buffer and were bubbled constantly with 21%O_2+5%CO_2 gas. After 10-15 min stabilization period, the baseline tension of the rings was adjusted to 1 g before all experiments. The artery rings were exposed to increasing doses of particles from 1 to100 μg/ml. The rings were washed with buffer between the two doses of particles. The maximum tension within 5 min after each dose of particles was recorded.

Cultured human pulmonary artery endothelial cells (HPAECs). HPAECs were grown in endothelial growth medium (EGM-2) supplemented with fetal bovine serum 2 %,

hydrocortisone 0.04 %, human fibroblast growth factor-B 0.4 %, vascular endothelial frowth factor 0.1 %, R^3-insulin growth factor-1 0.1 %, ascorbic acid 0.1 %, human epithelial growth factor 0.1 %, GA-1000, 0.1 % and heparin 0.1 %. Cells at passages 4-9 grown to 80 % confluence in 6-well plates were used for the experiments. The cells were exposed to particles at 1-100 μg/ml for up to 20 min.

Western blot analysis. After the exposure, the cells were washed once with ice cold phosphate-buffered saline (PBS) and then lysed with RIPA buffer (1 % Nonidet P-40, 0.5 % sodium deoxycholate, and 0.1% SDS in PBS, PH7.4) containing 0.1 mM vanadyl sulfate and protease inhibitors (0.5 mg/mL aprotinin, 0.5 mg/mL E-64, 0.5 mg/mL pepstatin, 0.5 mg/mL bestatin, 10 mg/mL chymostatin, and 0.1 ng/mL leupeptin). The cell lysates were then centrifuged at 3,000 g for 10 min at 4 °C. Protein concentration of supernatant was measured with Bio-Rad protein assay reagent. Cellular proteins were separated by 10% SDS-PAGE and transferred to a polyvinylidene difluoride membrane. The blot was blocked with 5% milk in PBS with 0.05% Tween-20 for 1 h at room temperature, washed briefly, and then probed with primary antibodies against phospho-p38 or phosphor-ERK1/2 overnight at 4°C. This was followed by incubation with HRP-conjugated secondary antibodies. Bands were detected by using ECL and films. The blot was then stripped, and reprobed with antibodies against to total p38 or total ERK1/2 and appropriate HRP-conjugated secondary antibodies.

Statistical analysis. Data shown in text and figures are mean ± standard error (SE). Data from the artery ring experiments were analyzed by the repeat measures analysis of variance (ANOVA). Data from the cell experiments were analyzed by ANOVA followed

by the Scheffe's test for post-hoc comparisons. The statistical analysis was performed using commercially available software (Statview version 5.0.1, SAS Institute Inc., Cary, NC). A P value of < 0.05 was taken as statistically significant.

RESULTS

Effects of losartan on UP-induced pulmonary artery constriction. Treatment of isolated rat pulmonary artery rings with UP produced a dose-dependent increase in vasoconstriction (Figure 1A). At 100 μg/ml, the ring tension was approximately 25% of that produced by 1 μM of phenylephrine. UP-induced pulmonary vasoconstriction was inhibited by losartan (0.2 μM), an AT_1R receptor antagonist (Figure 1B).

Effects of UP on ERK1/2 and p38 phosphorylation. Figure 2A shows the time-dependent increase in phospho-ERK1/2 induced by UP. The intensity of phosphorylated ERK1/2 peaked at 5 min and gradually decreased. The phosphorylation of ERK1/2 was enhanced significantly by 1-100 μg/ml of UP (Figure 2B). The phosphorylated p38 increased with time after UP treatment (Figure 2C). There was also a dose-dependent increase in the intensity of phospho-p38 (Figure 2D). The UP-induced phosphorylation of ERK1/2 and p38 was completely inhibited by PD98059, an ERK1/2 MAPK inhibitor, and SB203580, a p38 MAPK inhibitor (Figure 3A and 3B). The UP-induced constriction of rat pulmonary artery was also attenuated by PD98059 and SB203580 (Figure 3C and 3D), indicating that activation of ERK1/2 and p38 may mediate UP-induced pulmonary vasoconstriction.

Effects of losartan on UP-induced ERK1/2 and p38 phosphorylation. We then investigated the role of losartan in UP-induced ERK1/2 and p38 phosphorylation. HPAEC were pretreated with losartan (0.2 μM) for 30 min before incubation with 10 μg/mL UP for 10 min. Figure 4 shows that the UP-induced ERK1/2 and p38

phosphorylation was completely inhibited by losartan, indicating that AT_1R mediates the activation of ERK1/2 and p38 MAPK induced by UP.

Effects of particle components on ERK1/2 and p38 phosphorylation. Since the soluble fraction of the particles may better penetrate the alveolar-capillary membrane and be in contact with the pulmonary vessels than the whole particles when inhaled, we determined whether or not the soluble fraction of UP could activate ERK1/2 and p38 MAPK. Figure 5A and 5B show that the water-soluble fraction of UP was capable of increasing the phosphorylation of ERK1/2 and p38. The insoluble fraction was equally effective. Similar to the whole UP particles, the soluble fraction-induced activation of ERK1/2 and p38 was inhibited by losartan (Figure 5C and 5D).

Effects of UP-associated soluble metals on pulmonary artery constriction. To determine which water-soluble metal components of UP may be responsible for the activation of ERK1/2 and p38 MAPK, we tested the vasoconstrictor effects of soluble metals contained in UP. We found that copper (Cu) and vanadium (V) induced significant vasoconstriction (Figure 6). Other metals, including nickel, iron, manganese, zinc and aluminum, produced no or weak vasoconstriction (data not shown).

Effect of Cu and V on ERK1/2 and p38 phosphorylation. Since only Cu and V showed significant vasoconstrictor activity, we determined whether or not Cu and V could activate ERK1/2 and p38 MAPK. Figure 7 shows that $CuSO_4$ and $VOSO_4$ increased phosphorylation of ERK1/2 and p38 in a dose-dependent manner.

Effects of losartan on copper- and vanadium-induced phosphorylation of ERK1/2 and p38. We further determined whether or not the Cu- and V-induced activation of ERK1/2 and p38 was inhibitable by losartan. Figure 8 shows that Cu- and V-induced phosphorylation of p38 was inhibited by losartan. The phosphorylation of ERK1/2, however, was not inhibited by losartan (data not shown).

Effects of captopril on UP-induced ERK1/2 and p38 phosphorylation. Since angiotensin II, the ligand for AT_1R, is a metabolic product of angiotensin-converting enzyme (ACE), we further determined whether or not UP-induced AT_1R mediated MAPK activation required ACE. We pretreated the cells with captopril (100 μM), an ACE inhibitor, for 30 min before the addition of UP. Figure 9 shows that the UP-induced ERK1/2 and p38 phosphorylation was inhibited by captopril.

DISCUSSION

In this study, we showed that UP induced vasoconstriction in rat pulmonary arterial rings. Thus UP joined a list of pollutant particles that have been shown to cause vasoconstriction, including CAPs (Batalha et al. 2002), residual oil fly ash (Huang et al. 2002) and diesel particles (Tzeng et al. 2003). We further showed that the UP-induced pulmonary vasoconstriction could be inhibited by losartan, an AT_1R antagonist, indicating that the renin-angiotensin system may play an important role. PM has been shown to activate several vasoactive pathways, including the release of the endothelins (Bouthillier et al. 1998; Thomson et al. 2004), the activation of EGFR (Huang et al. 2002) and the inhibition of NO production (Bai et al. 2001; Bouthillier et al. 1998; Huang et al. 2002; Ikeda et al. 1995). Compared to these vasoactive pathways, the angiotensin II-AT_1R pathway has the following advantages: 1) the activation of AT_1R-mediated signaling by particles occurs earlier than that of endothelins, 2) angiotensin II is a much more potent vasoconstrictor than EGF and 3) activation of AT_1R leads to vasoconstriction rather than the loss of vasodilator activity as a result of inhibition of NO production. In these aspects, the AT_1R pathway may be a more clinically relevant mechanism for the acute cardiovascular events associated with ambient PM demonstrated in the epidemiological and clinical studies, especially in patients with compromised heart function.

Our study also showed that both the water soluble and insoluble fractions of UP were equally effective in increasing ERK1/2 and p38 phosphorylation. The effects by the water-soluble fraction would be more biologically relevant because components in this fraction are more likely than the whole particles or the insoluble fraction to gain access to

the pulmonary vessels quickly by diffusion after the particles are inhaled into the lung. Many components are present in the water-soluble fraction of UP. Among them are transition metals. In this study, we found that Cu and V produced strong vasoconstriction and activated ERK1/2 and p38. Thus Cu and V may be among the active components for PM-induced vasoconstriction. Residual oil fly ash, a pollutant dust containing abundant V, also produces vasoconstriction and MAPK activation (Huang et al. 2002; Silbajoris et al. 2000). Cu and V may reach the vasculature via specialized membrane transporters (Chasteen et al. 1986; Eisses and Kaplan 2002). Note that V appeared to be a stronger inducer for ERK1/2 and p38 phosphorylation than Cu but its vasoconstrictor property was weaker. This indicated that V, a non-specific protein tyrosine phosphatase inhibitor, may also activate other signaling proteins that counteract vasoconstriction. Our results do not exclude other mechanisms that are metal-independent. Diesel particles, which contain little metal, impair endothelium-dependent vasorelaxation (Ikeda et al. 1995) possibly via the production of reactive oxygen species inactivating nitric oxide. Ultrafine particles (PM with a mass median aerodynamic diameter <100 nm), which can more effectively penetrate the distal lung regions, may produce cardiovascular adverse effects via more intense lung inflammation (Brown et al. 2000; Utell and Frampton 2000). Previous studies have also shown that some components of ultrafine particles deposited in the respiratory tract could enter the circulation exerting their adverse effects on cardiovascular system (Takenaka et al. 2001).

The UP-induced activation of ERK1/2 and p38 could be inhibited by an antagonist of AT_1R, losartan. Similar inhibition by losartan was also seen when activation was

induced by the water-soluble fraction. This is the first demonstration that PM-induced vascular effects may be mediated by the angiotensin signaling. AT_1R is one of the four G protein-coupled receptors that mediates intracellular signaling induced by angiotensin II (Touyz and Berry 2002). AT_1R activation leads to cell growth, vascular contraction, inflammatory responses and salt and water retention (Touyz and Berry 2002). These effects are regulated by complex intracellular signaling pathways including MAPKs, phospholipase C, protein kinase C and phospholipase A2. Our results that inhibitors of ERK1/2 and p38 MAPKs attenuated UP-induced vasoconstriction support the established role of these MAPKs in angiotensin II-induced vasoconstriction (Ishihata et al. 2002; Massett et al. 2002; Meloche et al. 2000; Touyz et al. 1999). Phosphorylation of MAPKs may further activate downstream MAPK-activated protein kinase-2 and heat shock protein 27 with or without phosphorylation of myosin light chain (Meloche et al. 2000; Roberts 2004). Occupancy and activation of AT_1R also stimulates many intracellular non-receptor tyrosine kinases, such as Src, Pyk2, p130Cas, FAK and JAK/STAT (Eguchi and Inagami 2000). In the cardiovascular system, alterations of these highly regulated pathways underlie the pathological processes such as hypertension and atherosclerosis, conditions that also have been linked to PM exposure (Ibald-Mulli et al. 2001; Suwa et al. 2002). That losartan only inhibited p38 activation induced by V and Cu indicates that other components of PM may also be involved in PM-induced ERK1/2 MAPK activation.

The mechanisms by which UP activates AT_1R are unclear. Since the UP-induced phosphorylation of ERK1/2 and p38 could be inhibited by captopril, an ACE inhibitor,

the ACE activity appeared required for these effects. The vascular endothelial cells contain a large amount of ACE and can synthesize angiotensins via the local renin-angiotensin system (Kifor and Dzau 1987). Such a local renin-angiotensin system is likely the more important source of angiotensin II in our cultured HPAEC than the extracellular angiotensin I contained in the culture medium. One might also consider whether or not UP may act as an AT_1R agonist. At least one non-peptide agonist of AT_1R has been described. One such compound is a biphenylimidazole derivative (L-162313), whose biological effects could be inhibited by AT_1R antagonist (Perlman et al. 1995). Binding of non-peptide agonist of AT_1R may lead to conformational modifications that affect the preferential binding of agonists or antagonists (Costa-Neto et al. 2002).

CONCLUSIONS

The renin-angiotensin system plays an important role in many types of inflammatory and cardiovascular diseases. Angiotensin II via AT_1R signaling has been shown to promote cell growth and regulate the expression of bioactive substances such as vasoconstrictor hormones, growth factors, cytokines, aldosterone and extracellular matrix components (Jacoby and Rader 2003; Schiffrin and Touyz 2004). Our *in vitro* results indicate that the angiotensin-AT_1R signaling pathway may be important in mediating vascular effects induced by PM. Further studies are needed to determine the role of the circulating and local renin-angiotensin systems in PM-induced adverse cardiovascular effects.

DISCLAIMER

The research described in this article has been reviewed by the Health Effects and Environmental Research Laboratory, United States Environmental Protection Agency and has been approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency, nor does mention of the trade names or commercial products constitute endorsement or recommendation for use.

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FIGURE LEGENDS

Figure 1. Constriction of isolated rat pulmonary artery induced by urban particles (A) and the effects of losartan, an angiotensin II receptor subtype 1 antagonist, on urban particle (UP)-induced vasoconstriction (B). The constriction was measured by ring tension and expressed as % maximum tension induced by 1 μM of phenylephrine. *: $P < 0.05$ vs. 0.01 μg/ml; #: $P < 0.05$ vs. UP. N = 6-8 rings from different animals.

Figure 2. Effects of urban particle (UP) on phosphorylation of p38 and ERK MAPKs. (A) Time-dependent increase in phospho-ERK1/2 (p-ERK1/2). Confluent cells were treated with 10 μg/mL of UP for up to 20 min. (B) Dose-dependent increase in p-ERK1/2. Cells were treated with 1, 10 and 100 μg/ml of UP for 10 min. (C) Time-dependent increase in phospho-p38 (p-p38). Confluent cells were treated with 10 μg/mL of UP for up to 20 min. (D) Dose-dependent increase in p-p38. Cells were treated with 1, 10 and 100 μg/ml of UP for 10 min. Fifty μg cell lysates were separated by 10% SDS-PAGE gel and immunoblotted with monoclonal antibodies against phospho-p38 or p-ERK1/2. The blot was then stripped and re-probed with antibodies against total p38 or ERK. Results are representative blots of three independent experiments.

Figure 3. Effects of PD98059, an ERK1/2 MAPK inhibitor and SB203580, a p38 MAPK inhibitor on urban particle (UP)-induced phosphorylation of ERK1/2 (A) and p38 (B) respectively. Quiescent cells were pretreated with PD98059 (30 μM) and SB203580 (10 μM) for 30 min and then incubated with 10 μg/mL UP for 10 min. Phosphorylation of p38 and ERK in cell lysates were measured by Western blotting. The figure is representative of 3 independent experiments. The effects of PD98059 (30 μM) and

SB203580 (10 μM) on UP-induced pulmonary vasoconstriction are shown in (C) and (D) respectively. N = 4-6 rings from different animals.

Figure 4. Effects of losartan on urban particle (UP)-induced phosphorylation of p38 and ERK. Quiescent cells were pretreated with losartan (an angiotensin receptor type 1 antagonist, 0.2 μM) for 30 min and then incubated with 10 μg/mL UP for 10 min. Phosphorylation of p38 and ERK in cell lysates were measured by Western blotting. A representative Western blot (upper panel) and the densitometry results (lower panel) are shown. Data are mean ± standard error (SE). N = 4 independent experiments. *: $P< 0.05$.

Figure 5. (A) and (B): Phosphorylation of ERK and p38 by the components of urban particles (UP). The cells were treated with the whole UP (10 μg/ml), and the water-soluble fraction (sol-UP) and insoluble fraction (Ins-UP) extracted from 10 μg/ml of UP for 10 min. (C) and (D): Effects of losartan on phosphorylation of ERK and p38 induced by the soluble fraction of urban particles (sol-UP). A representative Western blot from 3 independent experiments is shown. . N = 3 independent experiments. *: $P< 0.05$ vs. control (ctr); #: $p < 0.05$ vs. Sol-UP.

Figure 6. Effects of copper and vanadium on pulmonary vasoconstriction. The constriction was measured by ring tension and expressed as % maximum tension induced by 1 μM of phenylephrine. *: $P < 0.05$ vs. control. N = 6-8 rings from different animals.

Figure 7. Effects of copper and vanadium on phosphorylation of ERK and p38. Cells were treated with copper sulfate and vanadyl sulfate for 10 min. Representative western blots (upper panel) and the densitometry results (lower panel) are shown. N = 4 independent experiments.

Figure 8. Effects of losartan on (A) copper- and (B) vanadium-induced p38 phosphorylation. Cells were pretreated with losartan (an AT_1R inhibitor, 0.2 μM) for 30 min before incubation with copper sulfate (0.1 μM) or vanadyl sulfate (0.25 μM) for 10 min. A representative western blot and densitometry results are shown. N = 4 each. *: $P < 0.05$ vs. control; #: $P < 0.05$ vs. UP.

Figure 9. Effects of captopril on urban particle (UP) induced ERK1/2 and p38 phosphorylation. Cells were pretreated with captopril (an angiotensin-converting enzyme inhibitor, 100 μM) for 30 min before incubation with UP (10 μg/ml) for 10 min. A representative western blot and densitometry results are shown. N = 4 each. *: $P < 0.05$ vs. control; #: $P < 0.05$ vs. UP.

Figure 1

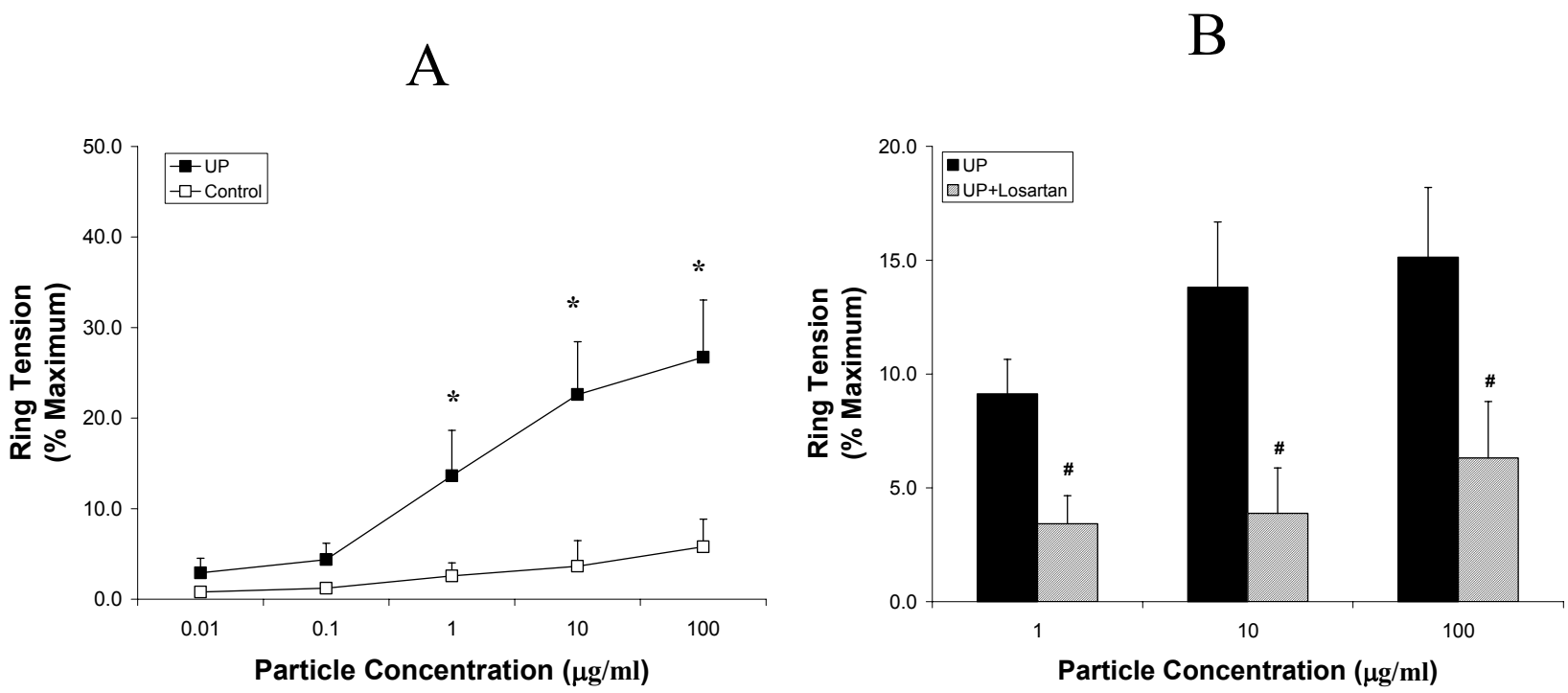

A
B
UP
Control
UP+Losartan
Ring Tension (% Maximum)
Particle Concentration (μg/ml)
0.0
10.0
20.0
30.0
40.0
50.0
5.0
15.0
0.01
0.1
1
10
100
*
#

Figure 2

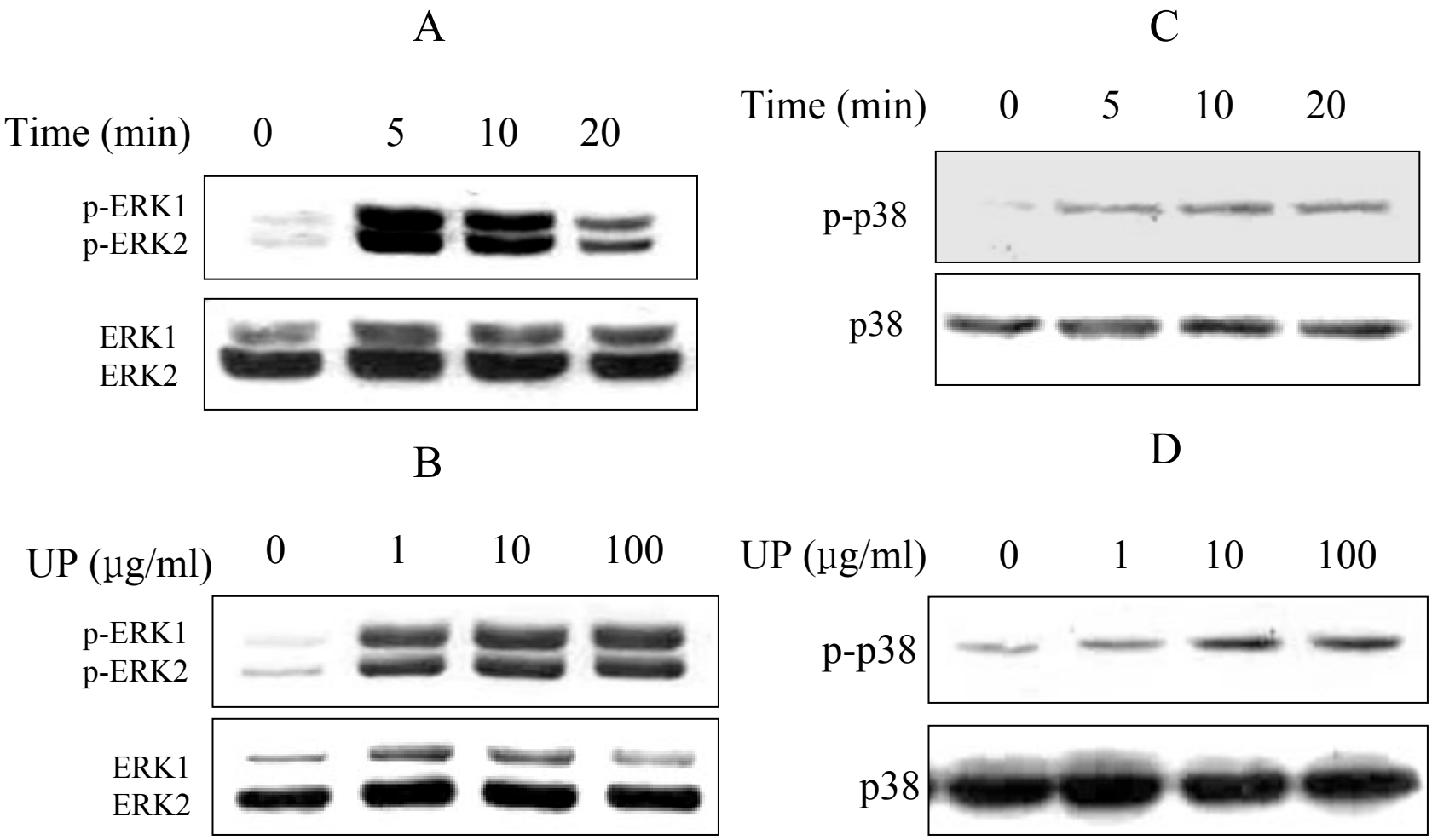

A
Time (min) 0 5 10 20
p-ERK1
p-ERK2
ERK1
ERK2
C
Time (min) 0 5 10 20
p-p38
p38
B
UP (μg/ml) 0 1 10 100
p-ERK1
p-ERK2
ERK1
ERK2
D
UP (μg/ml) 0 1 10 100
p-p38
p38

Figure 3

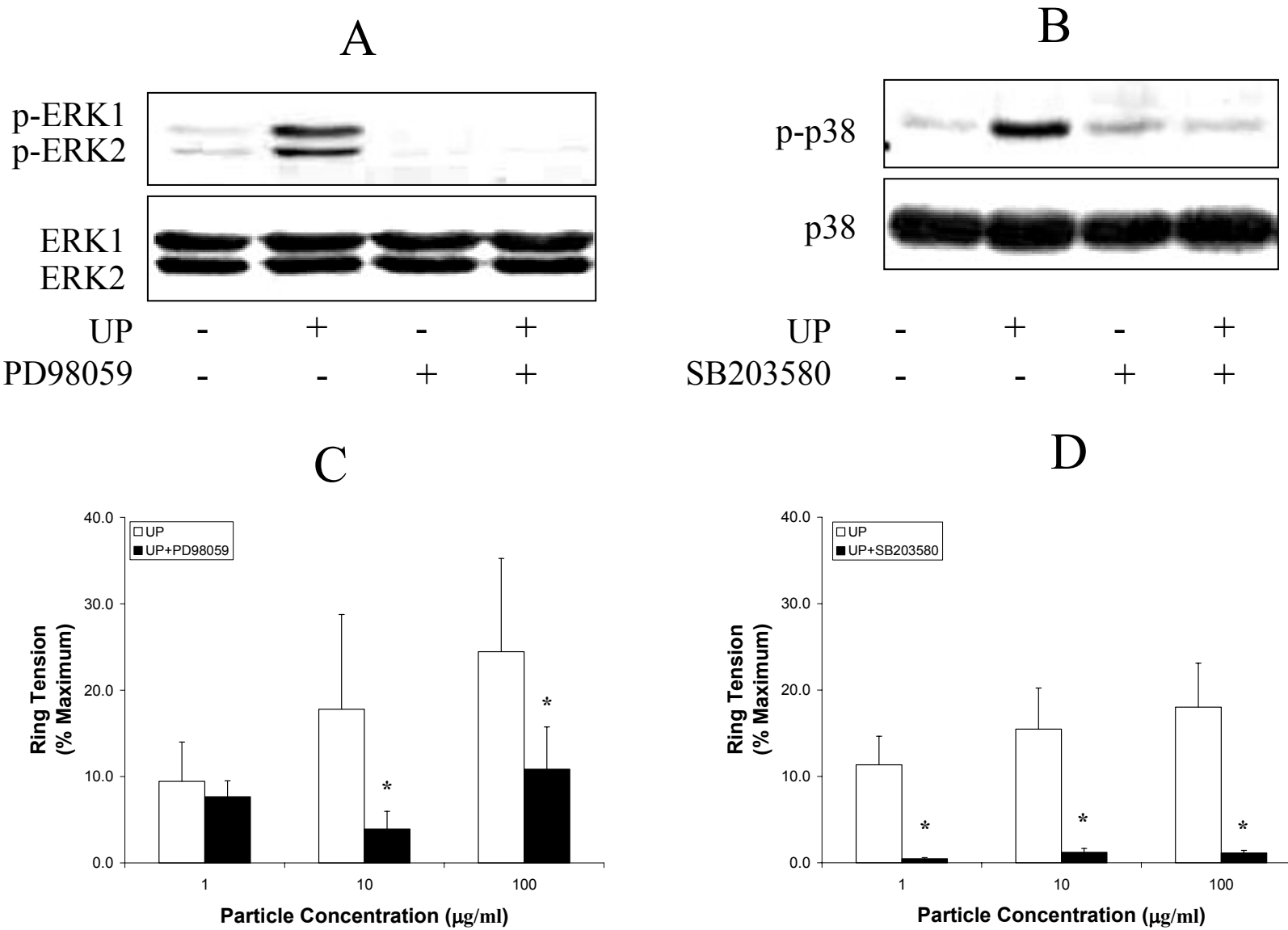

A
p-ERK1
p-ERK2
ERK1
ERK2
UP - + - +
PD98059 - - + +
B
p-p38
p38
UP - + - +
SB203580 - - + +
C
UP
UP+PD98059
Ring Tension (% Maximum)
40.0
30.0
20.0
10.0
0.0
1
10
100
Particle Concentration (μg/ml)
*
D
UP
UP+SB203580
Ring Tension (% Maximum)
40.0
30.0
20.0
10.0
0.0
1
10
100
Particle Concentration (μg/ml)
*

Figure 4

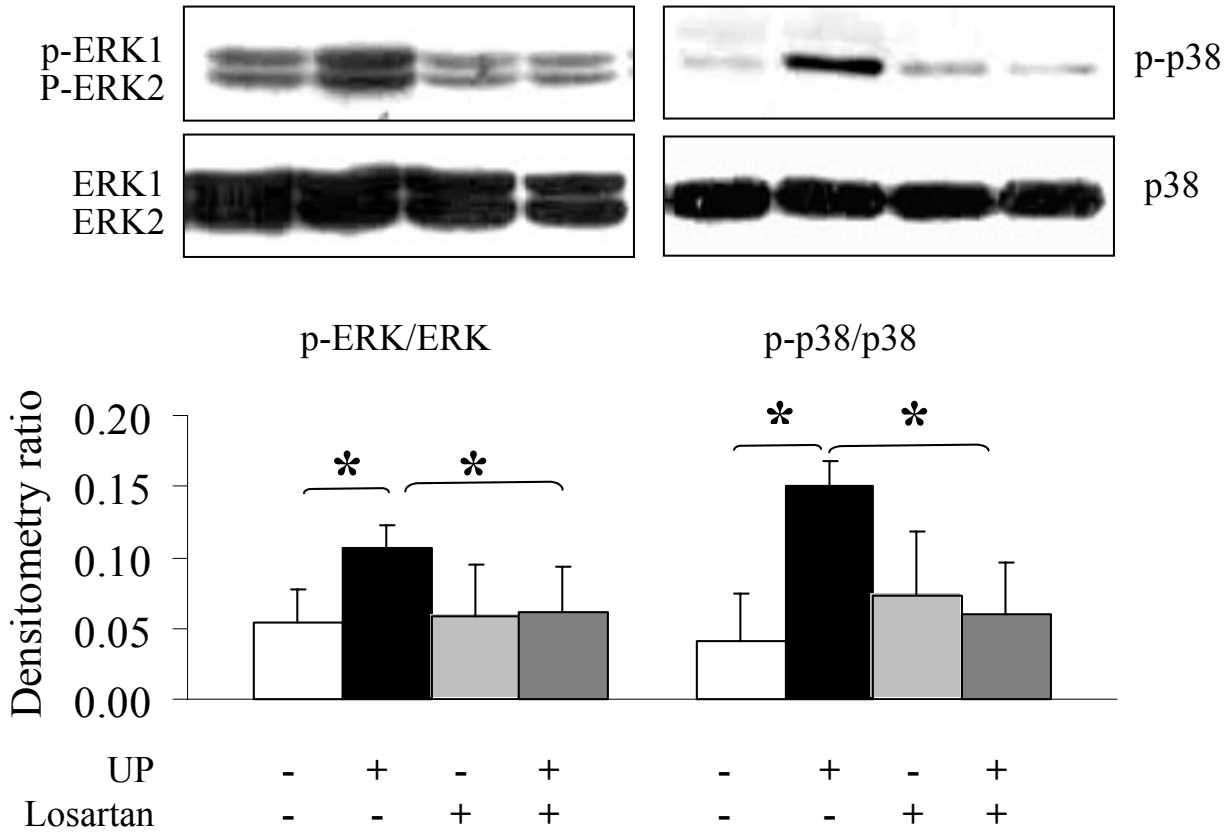

p-ERK1
P-ERK2
ERK1
ERK2
p-p38
p38
p-ERK/ERK
p-p38/p38
Densitometry ratio
0.20
0.15
0.10
0.05
0.00
*
*
*
*
UP
Losartan
- + - +
- - + +
- + - +
- - + +

Figure 5

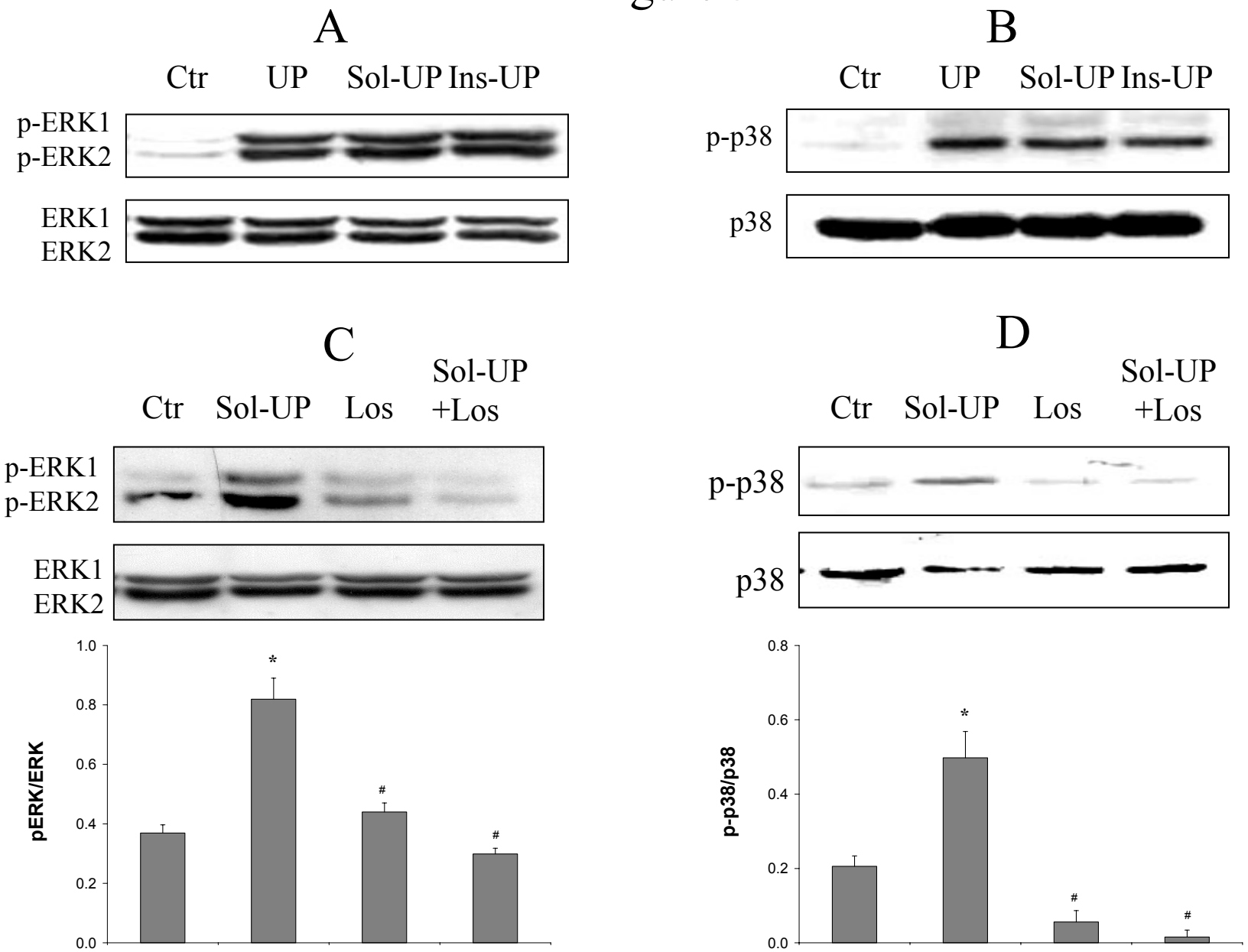

A
Ctr
UP
Sol-UP
Ins-UP
p-ERK1
p-ERK2
ERK1
ERK2
B
Ctr
UP
Sol-UP
Ins-UP
p-p38
p38
C
Ctr
Sol-UP
Los
Sol-UP +Los
p-ERK1
p-ERK2
ERK1
ERK2
pERK/ERK
1.0
0.8
0.6
0.4
0.2
0.0
*
#
#
D
Ctr
Sol-UP
Los
Sol-UP +Los
p-p38
p38
p-p38/p38
0.8
0.6
0.4
0.2
0.0
*
#
#

Figure 6

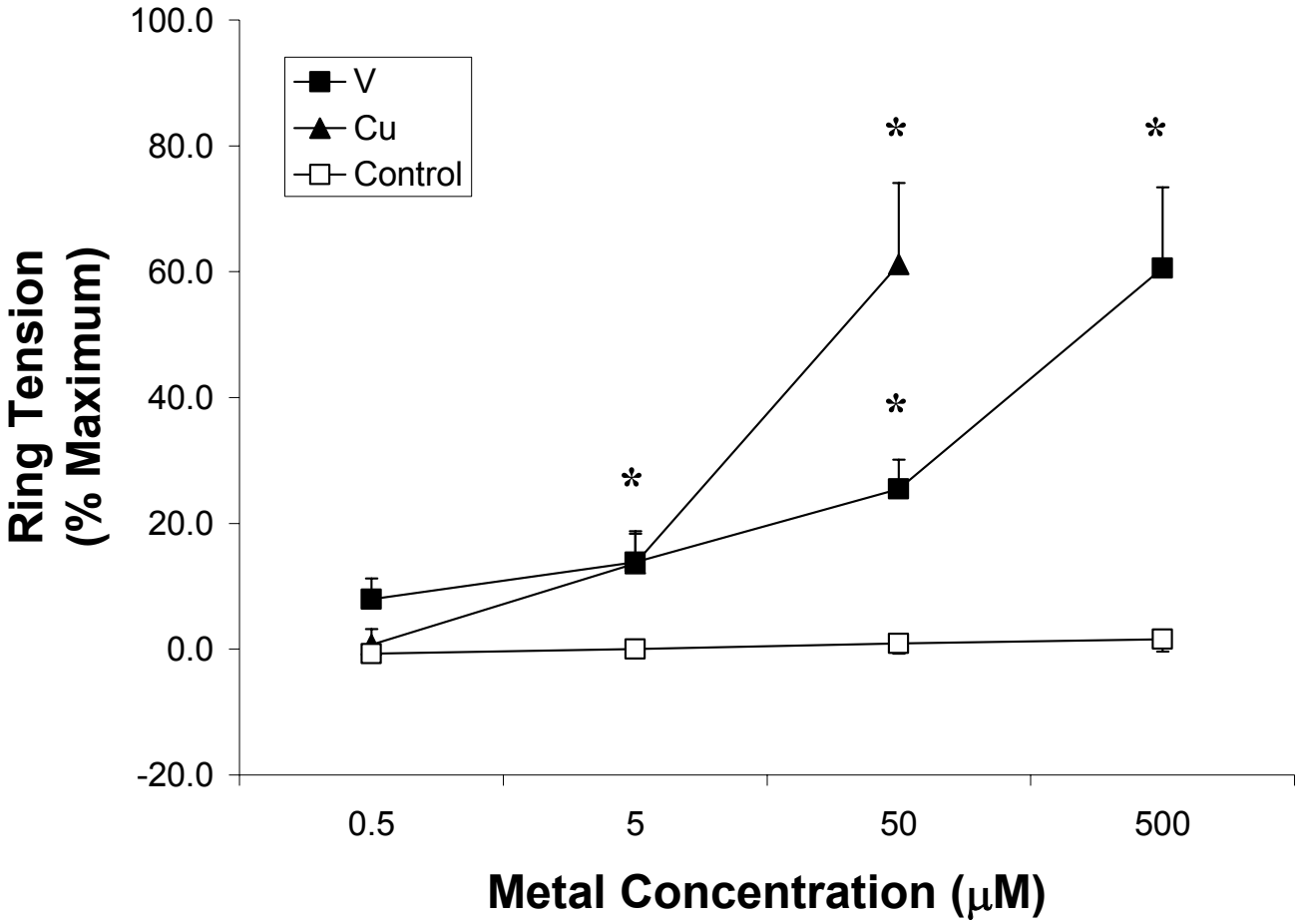
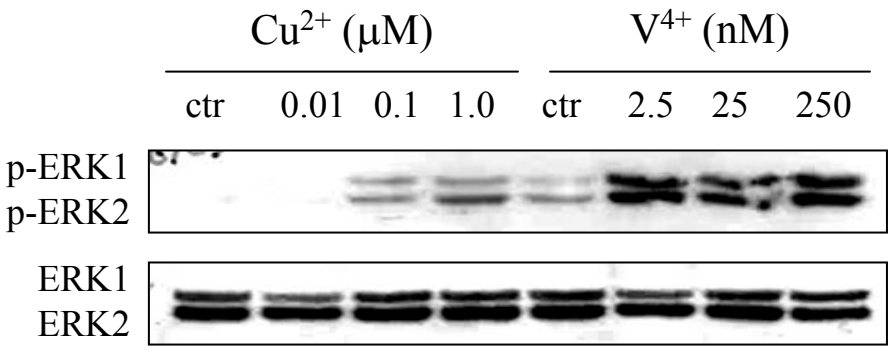
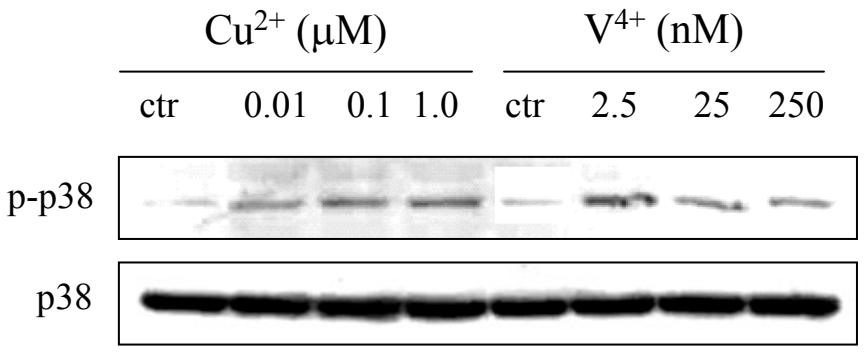
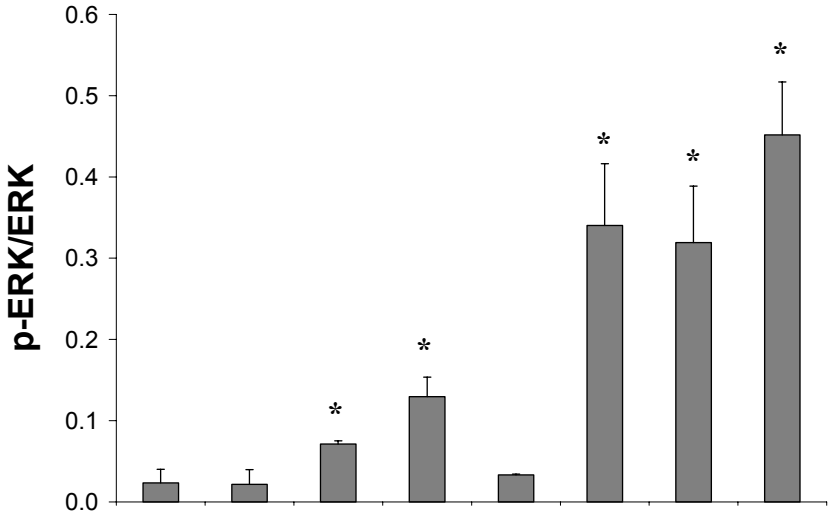

100.0
80.0
60.0
40.0
20.0
0.0
-20.0
Ring Tension
(% Maximum)
V
Cu
Control
*
*
*
0.5
5
50
500
Metal Concentration (μM)

Figure 7


Cu2+ (μM)
V4+ (nM)
ctr
0.01
0.1
1.0
ctr
2.5
25
250
p-ERK1
p-ERK2
ERK1
ERK2


Cu2+ (μM)
V4+ (nM)
ctr
0.01
0.1
1.0
ctr
2.5
25
250
p-p38
p38


p-ERK/ERK
0.0
0.1
0.2
0.3
0.4
0.5
0.6
*

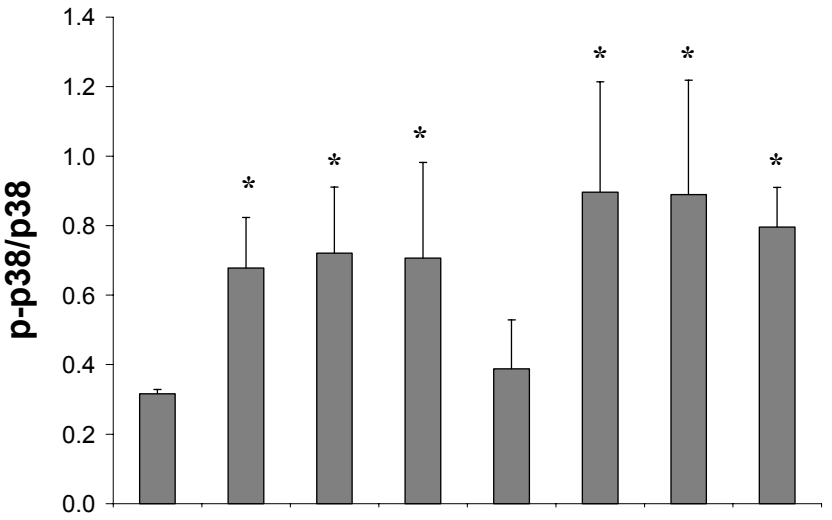

p-p38/p38
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
*

Figure 8

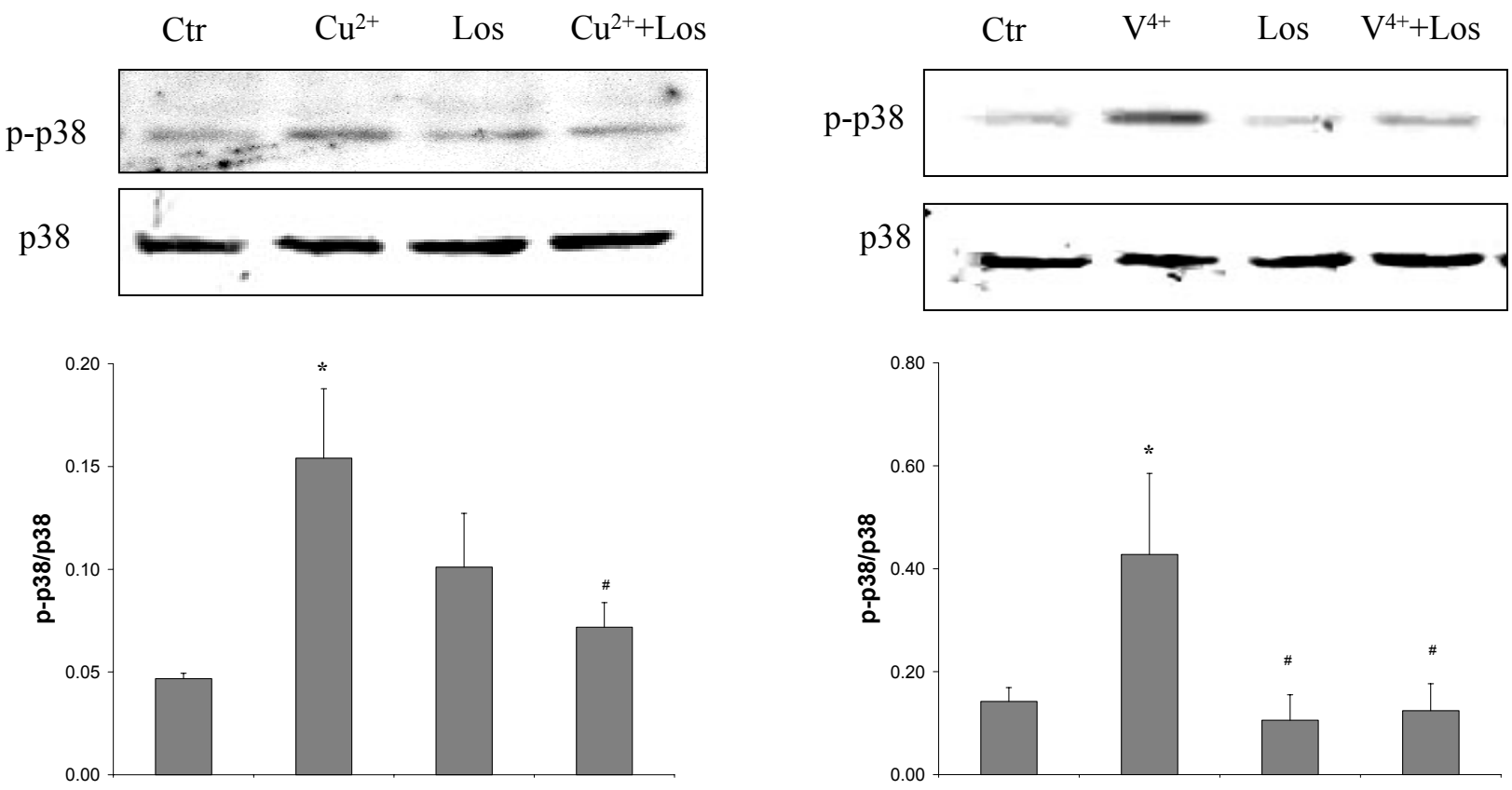
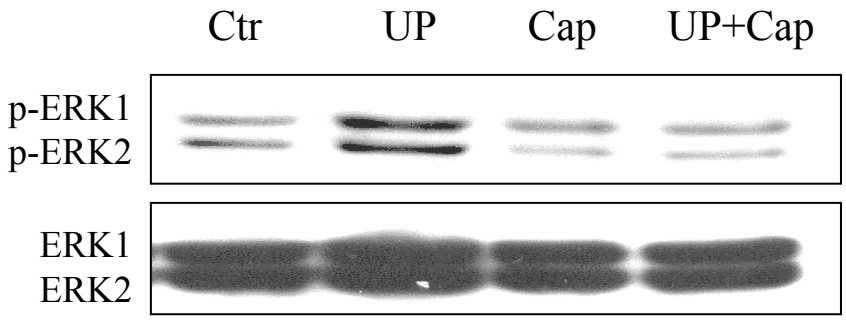
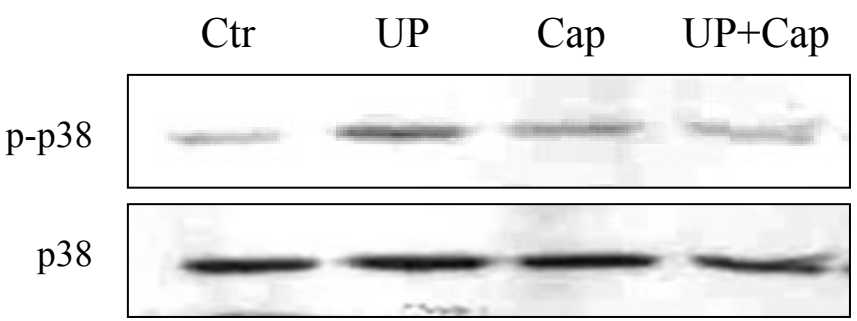
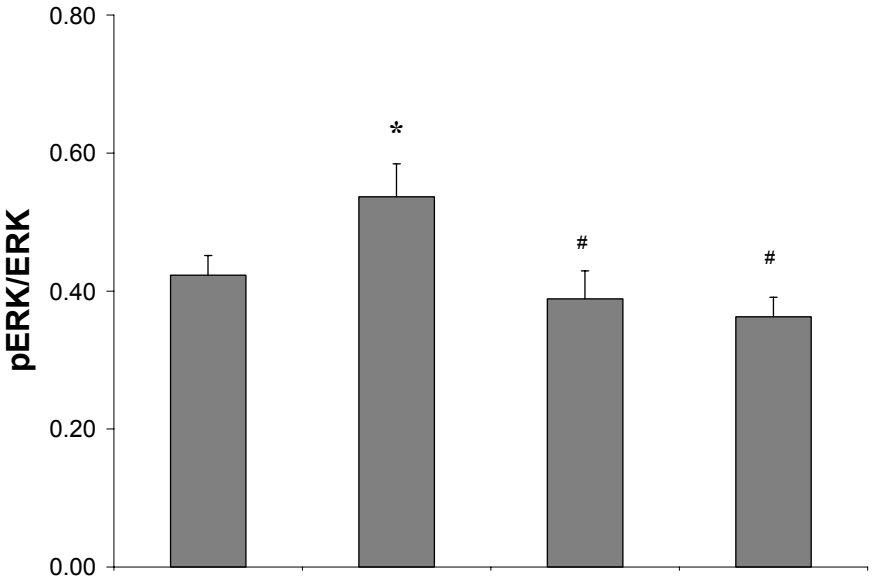
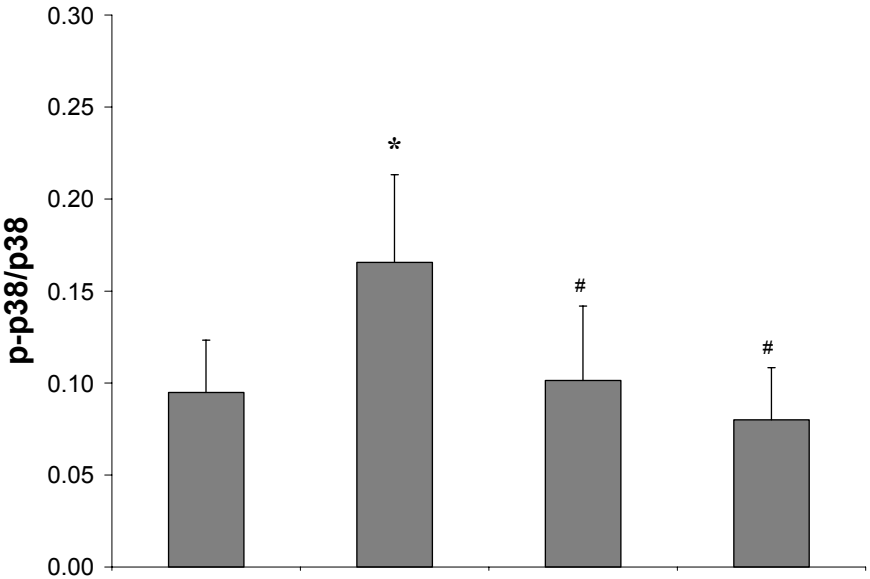

A
B
Ctr
Cu2+
Los
Cu2+ +Los
V4+
V4+ +Los
p-p38
p38
p-p38/p38
0.20
0.15
0.10
0.05
0.00
0.80
0.60
0.40
0.20
0.00
*
#

Figure 9


Ctr
UP
Cap
UP+Cap
p-ERK1
p-ERK2
ERK1
ERK2


Ctr
UP
Cap
UP+Cap
p-p38
p38


pERK/ERK
0.80
0.60
0.40
0.20
0.00
*
#
#


p-p38/p38
0.30
0.25
0.20
0.15
0.10
0.05
0.00
*
#
#